

IT IS CLAIMED:

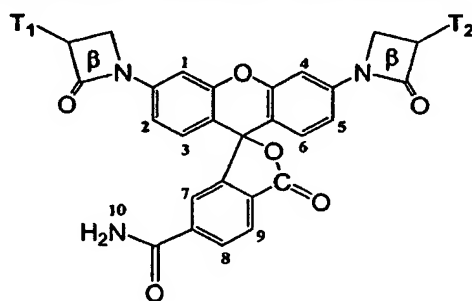
1. A multiplexed assay for monitoring the level of transcription of one or more genes in response to one or more potential regulatory stimuli,
5 comprising:
 - placing transfected cells in each of a plurality of wells, where the cells in each well are transfected with a genetic construct comprising a selected promoter operatively linked to the coding sequence for an enzyme having a selected enzymatic activity;
 - 10 adding to the cells in each well a probe selected from a set of probes, where each probe in the set is cleavable by the enzyme into a substrate moiety and an electrophoretic tag (e-tag) reporter having a detection group and a separation modifier that confers on the e-tag reporter, a unique electrophoretic mobility with respect to the e-tag reporters derived from the other probes in the
15 set;
 - incubating the cells and associated probes while exposing the cells to a potential regulatory stimulus;
 - obtaining the tags from the cells;
 - electrophoretically separating the combined tags; and
 - 20 determining, from the electrophoretic mobility and level of detection group of each separated e-tag reporter, the level of transcriptional response of each cell to the potential regulatory stimulus to which the cells were exposed.
2. The method of claim 1, wherein said incubating is carried out in
25 separate wells, and said obtaining includes combining the cells from separate wells, and obtaining said tags from the combined cells.
3. The method of claim 2, wherein the potential regulatory stimuli include one or more test compounds, and said exposing includes adding the
30 test compound(s) to the individual cell-containing wells.

plurality of different constructs, each comprising one of said promoters operatively linked to said coding sequence.

9. The method of claim 1, wherein the enzyme is selected from the group consisting of β -lactamase, β -galactosidase, an esterase, a protease, and a nuclease.

10. The method of claim 1, wherein each probe in the set further comprises a transport moiety that facilitates transport of the probe into a cell, and a transport moiety linkage that is subject to cleavage within the cell to release the transport moiety and thereby inhibit probe transport of the probe out of the cell.

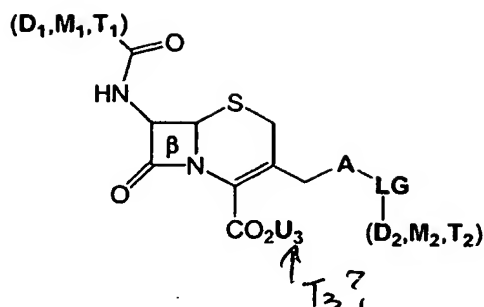
11. The method of claim 10, wherein said enzyme is β -lactamase, and one or more of the probes of the set has the form:



where exemplary positions of transport moieties are shown as T_1 and T_2 ; a substrate for the enzyme, S, is exemplified as a four-member β -lactam ring, labeled β ;

the main part of the structure comprises a fluorescein derivative, wherein various positions are numbered that are subject to chemical modification; and a separation modifier, M, (not shown) is present at one of the numbered positions.

12. The method of claim 10, wherein said enzyme is β -lactamase, and one or more of the probes of the set has the form:



where exemplary positions of transport moieties are shown as T_1 , T_2 and T_3 ;

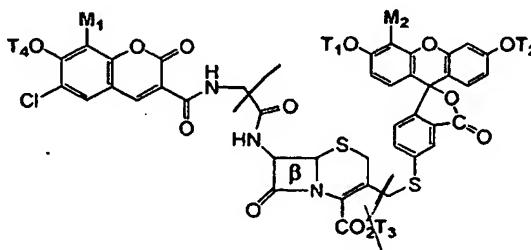
- 5 exemplary positions of detection groups are shown as D_1 and D_2 ;
 exemplary positions of separation modifiers are shown as M_1 and M_2 ;
 and

 a substrate for the enzyme, S, is exemplified as a four-member β -lactam ring, labeled β , wherein cleavage of the ring induces electron flow towards the
 10 acceptor, shown as A, causing cleavage of the probe by elimination of the leaving group, shown as LG.

13. The method of claim 12, wherein
 the probes comprise fluorophores at positions D_1 and D_2 ;
 15 the two fluorophores are capable of exhibiting efficient fluorescence resonance energy transfer; and
 cleavage of the β -lactam ring results in separation of the two fluorophores, thereby restoring fluorescent emission from the shorter wavelength fluorophor.

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14. The method of claim 13, wherein one or more of the probes of the set have the form:



where exemplary positions of the transport moiety are shown as T₁, T₂, T₃ and T₄;

the first detection group D₁ is exemplified as a cephalosporin;

5 the second detection group D₂ is exemplified as a fluorescein;

exemplary positions of the separation modifier are shown as M₁ and M₂;

and

the substrate is exemplified as a four-member β -lactam ring, labeled β .

10 15. The method of claim 1, wherein said detection group is a fluorescent moiety.

16. The method of claim 1, wherein said detection group includes a catalytic moiety capable of catalyzing a detectable reaction.

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17. The method of claim 1, wherein the probes in the set have the form (D, M_j) - S, where

(D, M_j) is the detection group D linked to the separation modifier M_j having a unique separation characteristic for each probe j in the set;

20 S is a substrate for the enzyme; and

the action of the enzyme on the probe produces an e-tag reporter of the form (D, M) - S', where S' is the residue of the substrate remaining with the e-tag reporter after reaction of S with the enzyme.

25 18. The method of claim 1, for determining the extent of interaction of a first hybrid protein having a DNA-binding domain that binds to the selected promoter, and a first interaction domain; and a second hybrid protein having a transcriptional activation domain and a second interaction domain that is to be tested for interaction with the first interaction domain; where (i) said promoter is
30 capable of activation by a polypeptide having a transcriptional activation domain when the transcriptional activation domain is in sufficient proximity to the gene, (ii) cell contains said first and second hybrid proteins, and (iii) said